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Research Article

Tracing the rise of silver carp (*Hypophthalmichthys molitrix*) in Thailand with eDNA

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Abstract

Silver carp (*Hypophthalmichthys molitrix*) is a filter-feeding cyprinid introduced globally for aquaculture that poses significant ecological risks to river systems worldwide. In Thailand, its distribution in the Chao Phraya River Basin has remained poorly documented due to limitations of traditional monitoring. This study applied environmental DNA (eDNA) analysis at 39 sites across the Ping, Wang, Yom, Nan, and Chao Phraya rivers during 2019, 2021, and 2023. Detection rates increased from 31% in 2019 to 59% in 2023, with eDNA detected at all sites by the final survey year. Bayesian occupancy modelling revealed moderate occurrence probabilities (ψ = 0.40–0.51) and consistently high laboratory detection reliability ($p_{11} \approx 0.90$). Temperature was the strongest predictor of detection efficiency, whereas other water-quality parameters had minimal effects on ψ, suggesting that environmental factors mainly influenced detectability rather than species presence. Comparison with Department of Fisheries records showed a contrast between long-term declines in Chinese carp catch and increasing eDNA detections of *H. molitrix*, indicating continued establishment and expansion of this species. Because official data still combine *H. molitrix* and bighead carp (*Hypophthalmichthys nobilis*) under one category, eDNA provides the species-level resolution needed to track individual taxa. These results demonstrate that molecular surveillance complements traditional fisheries assessments by offering finer spatial and temporal resolution for detecting introduced species, supporting the integration of eDNA techniques into regional biosecurity programmes.

Key words: Aquatic ecosystems, biological invasions, DNA surveillance, freshwater monitoring, invasive species, molecular detection, sustainable development

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Introduction

Silver carp (*Hypophthalmichthys molitrix*) represents one of the world's most successful aquatic introductions, with established populations now documented across multiple continents following its introduction for aquaculture purposes. Native to the major river systems of East Asia, this cyprinid species was initially valued for its rapid growth rates and filter–feeding behaviour that can improve water quality in polyculture systems (Kolar et al. 2005). However, subsequent escapes from aqua-

culture facilities have led to widespread establishment in natural waterways, where silver carp now poses substantial ecological and economic threats.

The expansion history of silver carp in North America provides compelling evidence of its ecological impact potential, with extensive documentation supporting concerns about ecosystem disruption (Irons et al. 2007; Sampson et al. 2009; Coulter et al. 2016). Introduced to the United States during the 1970s and 1980s for private aquaculture and wastewater treatment applications, the species escaped containment during flood events in the 1990s and rapidly colonised the Mississippi River drainage system (U.S. Fish and Wildlife Service [USFWS], n.d.). Established populations have since disrupted native food webs through competition with indigenous planktivores, including freshwater mussels, larval fishes, and paddlefish (Irons et al. 2007; Coulter et al. 2016). The species exerts substantial pressure on the crucial link between primary production and intermediate trophic levels in large river systems, producing cascading effects throughout aquatic food webs (Sampson et al. 2009).

Beyond ecological impacts, silver carp presents unique management challenges due to its distinctive escape response, jumping up to three metres out of the water when startled by boat motors or other disturbances, creating safety hazards for recreational users (Kolar et al. 2007). Recognition of these multifaceted impacts led to silver carp being classified as an injurious species under federal regulation in the United States by 2007, highlighting the serious nature of establishment concerns (U.S. Fish and Wildlife Service 2007).

In Thailand, *H. molitrix* has been extensively cultivated in freshwater aquaculture systems and is commonly marketed alongside bighead carp (*Hypophthalmichthys nobilis*) under the collective designation "Chinese carps." While economically valuable, the potential for escapement from culture facilities into natural waterways raises serious conservation concerns, particularly within the ecologically critical Chao Phraya River Basin. This watershed system, encompassing the Ping, Wang, Yom, Nan, and main Chao Phraya rivers, represents Thailand's most important inland freshwater system, supporting exceptional biodiversity, intensive agricultural production, and major urban populations. The basin currently faces mounting environmental pressures from multiple introduced species, extensive habitat modification, and hydrological alterations associated with dam construction and water diversions (Dudgeon 2000).

Traditional fish monitoring approaches in Thailand rely primarily on fishery production statistics, localised surveys, and conventional capture–based sampling methodologies, approaches that have significant limitations for comprehensive species surveillance. While providing valuable baseline information, these approaches exhibit significant limitations for introduced species surveillance programmes. They frequently fail to detect low–density populations during critical early colonisation phases, suffer from taxonomic uncertainties when species are aggregated under common commercial names, and prove logistically challenging for comprehensive spatial coverage (Jerde et al. 2011; Civade et al. 2016). Current DOF records exemplify these constraints by reporting combined production and capture statistics for *H. molitrix* and *H. nobilis* under the general category of 'Chinese major carps,' without species-specific differentiation, limiting precise assessment of individual species distributions (Department of Fisheries Thailand 2019–2023).

Environmental DNA (eDNA) analysis has emerged as a valuable methodology for aquatic biodiversity assessment and species surveillance. Defined as genetic

material obtained directly from environmental samples without physical capture of target organisms, eDNA enables species detection through analysis of cellular material naturally shed into aquatic environments through normal metabolic processes, including mucus, faeces, gametes, and sloughed tissues (Ficetola et al. 2008; Deiner et al. 2017). This non–invasive approach has demonstrated particular effectiveness for detecting rare, cryptic, or invasive species across extensive spatial scales while providing cost–effective alternatives to traditional sampling methods (Lodge et al. 2012; Rees et al. 2014).

Despite its demonstrated potential, eDNA applications in Southeast Asian freshwater systems remain limited, representing a significant knowledge gap given the region's high vulnerability to biological invasions and extensive aquaculture development (Wee et al. 2023; Zhang et al. 2023). Recent studies have successfully demonstrated eDNA effectiveness for silver carp detection in North American systems, particularly through comprehensive surveillance programmes implemented in the Great Lakes basin since 2009 (Jerde et al. 2013; Nathan et al. 2014). However, systematic applications in tropical Asian watersheds remain largely unexplored.

This study presents a multi–year, basin–scale application of environmental DNA (eDNA) monitoring to track the spread of silver carp in Thailand's freshwater systems. Systematic water sampling was conducted at 39 strategically selected sites across the Chao Phraya River Basin during three survey periods: 2019, 2021, and 2023. We hypothesised that silver carp populations would increase over time, driven by the species' environmental plasticity and the expansion of aquaculture activities. Accordingly, our objectives were to: (a) assess spatiotemporal patterns in silver carp distribution using species–specific eDNA detection; (b) quantify occurrence and detection probabilities using Bayesian occupancy modelling; (c) identify environmental factors influencing detection outcomes through Bayesian variable selection; and (d) evaluate the effectiveness of eDNA monitoring compared to traditional fishery data. This integrated approach provides essential baseline information for silver carp management and offers a methodological framework for improving invasive species surveillance across Southeast Asia.

Methods

Study area

The Chao Phraya River Basin encompasses approximately 160,000 km^2 across central and northern Thailand, representing the country's largest and most economically important watershed system. The Chao Phraya River per se is 372 km in length, formed from its main upstream tributaries at Nakhon Sawan Province, and runs downward to enter the Gulf of Thailand (Deekrachang et al. 2024). The river is characterised by seasonal flooding, supporting the riparian ecosystems as well as aquatic floras and faunas along its vast floodplains (Park et al. 2021). The diversity of finfishes in this basin is reported at 328 species (Froese and Pauly 2025). We established 39 sampling locations distributed across five major river systems: Ping River (8 sites), Wang River (8 sites), Yom River (8 sites), Nan River (8 sites), and main Chao Phraya River (7 sites) (Suppl. material 1: table S1). Site selection balanced comprehensive spatial coverage with logistical accessibility (Fig. 1).

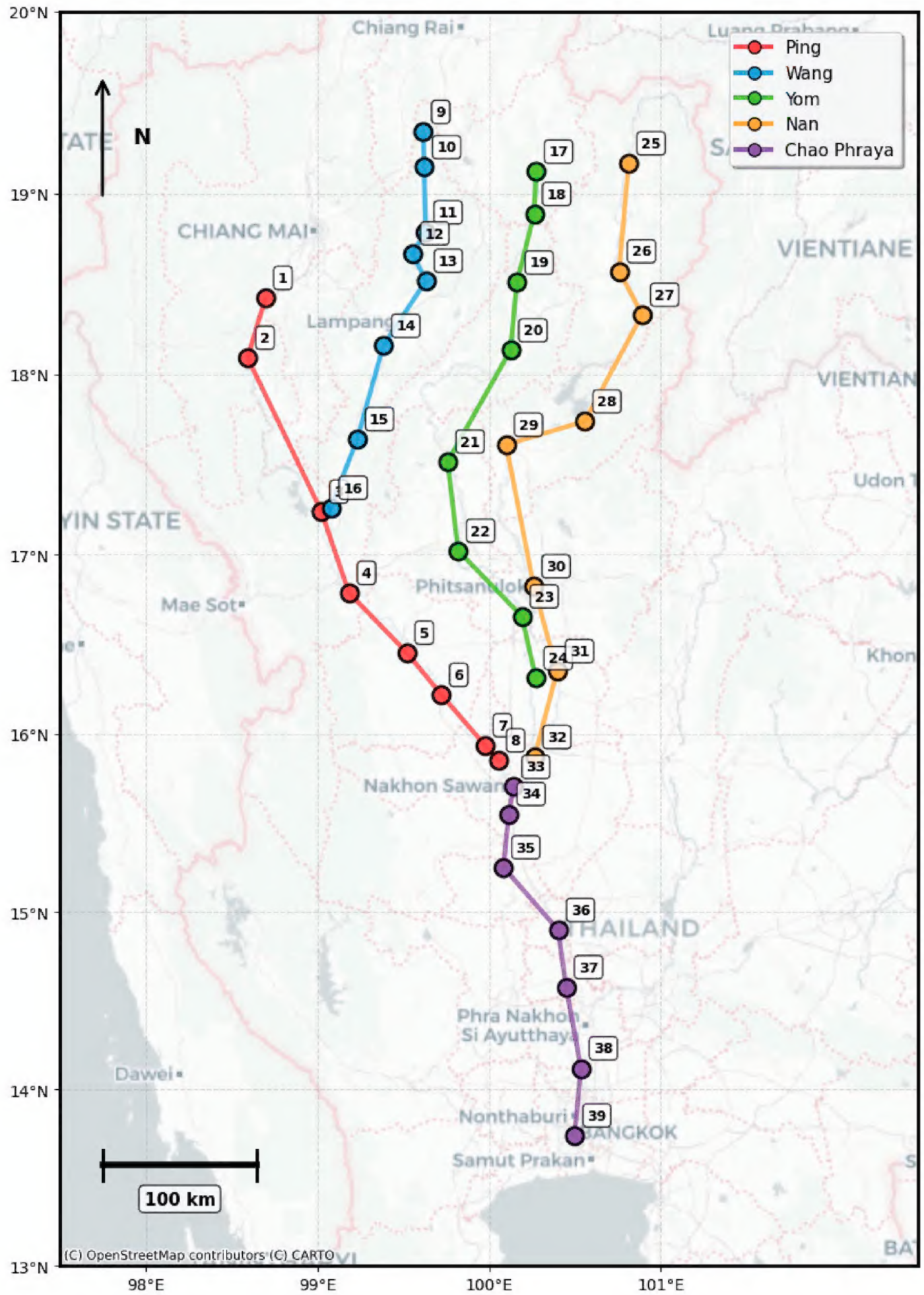

Figure 1. Location of 39 eDNA sampling sites across the Chao Phraya River Basin, Thailand.

eDNA sampling and extraction

Water samples were collected during three field campaigns conducted in November 2019, December 2021, and December 2023, corresponding to the post–monsoon period when river conditions are typically stable and conducive to eDNA detection.

All field equipment and sampling materials were prepared under strict contamination control protocols. Laboratory benches and reusable tools were decontaminated with 10% bleach and 70% ethanol, followed by two rinses with distilled water before and after each sampling batch. For fieldwork, equipment was prepared separately for each site. Syringes used for filtration were sterile, individually sealed by the manufacturer, and opened only immediately before use. Water-collection bottles were pre-labelled and assigned to a single site within each campaign; they

were never reused across different sites during the same sampling event. A new pair of sterile gloves was worn at every sampling site, and field staff avoided contact with surrounding substrates or water surfaces before collection. Field blanks consisting of sterile distilled water were exposed to the ambient environment at each sampling event and processed in parallel with environmental samples to monitor potential airborne or handling contamination. These procedures followed the general recommendations of Blackman et al. (2021) and Osathanunkul and Minamoto (2020), ensuring that cross-site contamination risk was minimised.

In total, 351 water samples were collected across the three–year study period (39 sites × 3 replicates × 3 years = 351 samples). At each sampling location, three replicate 1–L water samples were collected from the surface layer (0.5 m depth) using sterile polypropylene bottles. Sampling was conducted along the riverbank rather than mid-channel to allow consistent access across all sites and ensure safe and reproducible collection under variable flow conditions. Sampling at approximately 0.5 m depth along the bank minimises turbulence-related dilution while capturing representative eDNA from the main flow. Samples were processed immediately following collection by filtration through 0.7 µm glass fibre filters (Whatman GF/F) under sterile conditions. The DNA extraction was performed using DNeasy Blood & Tissue kits (Qiagen, Germany) with modifications following Osathanunkul and Minamoto (2020). To improve DNA yield and recovery from environmental filters, several modifications were made to the standard Qiagen protocol. The filter membranes were cut into small fragments using sterile scissors and incubated with 360 µL of ATL buffer and 40 µL of Proteinase K at 56 °C for 3 h with gentle agitation to maximise cell lysis. The amount of Proteinase K was doubled compared to the manufacturer's protocol to enhance digestion efficiency. After incubation, the lysate was transferred to a new tube, and the extraction proceeded according to the manufacturer's instructions. DNA was eluted twice in 50 µL aliquots of AE buffer (final volume = 100 µL) to increase total DNA recovery. After DNA extraction, each sample was purified using the OneStep PCR Inhibitor Removal Kit (Zymo Research, USA) to minimise the presence of substances that could interfere with PCR amplification. PCR inhibition was assessed using an internal positive control (IPC) consisting of a synthetic DNA fragment from the jellyfish *Chiropsoides buitendijki* (gBlocks™, Integrated DNA Technologies). This marine species does not occur in freshwater, making it suitable for use as an external control. A specific primer and probe set targeting the *C. buitendijki* 16S rRNA gene (forward primer 5'-CCCCAATCGAAATTAAGTTAGCC-3', reverse primer 5'-CACAGGTAGAGTGGAGAAATAGAG-3', and probe 5'-FAM-GTGAAGACGCAGCTTTGTCT-TAMRA-3') was used, and each reaction contained 1.5×10^2 copies of the synthetic template (Osathanunkul 2024). Inhibition was defined as a shift of three or more cycles in the IPC Cq value compared with the control reactions, following the method of Hartman et al. (2005). None of the extracts showed any indication of inhibition.

Environmental DNA analysis

Species-specific quantitative PCR (qPCR) assays targeting the *H. molitrix* mitochondrial NADH dehydrogenase subunit 2 (ND2) were performed using previously validated primers and probe developed by Farrington et al. (2015). The SC-TM5 primer–probe set was used, consisting of Forward 5'-CCACAACTTACCCTCCTTGCC-3', Reverse 5'-AAGGGTATTAATTTTTGTGGTGGA-3', and

Probe 5'-FAM-TCATGACATCCGCAGCATTCCTC-TAMRA-3'. Each sample was analysed in triplicate, with both positive and negative controls included in every run to ensure analytical reliability. The qPCR reactions contained 10.0 µL of TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific), 2.0 µL of DNA template, 900 nM of each primer, and 125 nM of probe in a total volume of 20 µL. Thermal cycling was conducted on a Rotor-Gene Q MDx 5plex system (Qiagen, Germany) under the following conditions: 95 °C for 10 minutes, followed by 50 cycles of 95 °C for 15 seconds and 60 °C for 1 minute.

Each qPCR run included both positive and negative controls to verify assay performance and monitor potential contamination. For positive controls, tissue-derived genomic DNA from *H. molitrix* was used to confirm assay efficiency and sensitivity. Negative controls were applied at three levels: (i) field negatives (sterile water handled and filtered alongside environmental samples), (ii) extraction blanks (AE buffer processed through the entire extraction workflow), and (iii) no-template controls (NTCs) containing molecular-grade water on each qPCR plate. No amplification occurred in any negative control, confirming that contamination was effectively prevented.

The limit of detection (LOD) and limit of quantification (LOQ) were determined following the framework of Hunter et al. (2017) and Klymus et al. (2020). The LOD was defined as the lowest concentration at which at least 95% of PCR replicates yielded amplification, while the LOQ represented the lowest concentration at which DNA copy number could be estimated with acceptable precision, corresponding to a coefficient of variation of ≤35%. A 10-fold serial dilution series ranging from 1.5×10^{6} to 1.5×10^{-3} copies per reaction, generated from a synthetic DNA gBlock® Gene Fragment (Integrated DNA Technologies, USA), was used to estimate these thresholds. In this study, amplification signals above the LOD were interpreted as detectable, whereas those below the LOQ were treated as below-quantification (bq) detections, representing target DNA presence at concentrations too low for reliable quantification. Samples showing no amplification across all replicates were considered non-detections.

Quantification cycle (Cq) values were converted to target concentrations using standard curves generated from synthesised target gene fragments. Copy numbers were first calculated separately for each technical replicate, and the mean value across the three replicates was used as the final eDNA concentration for that sample. This framework was applied consistently throughout the analysis and interpretation of results.

Assay specificity was validated through both *in silico* and *in vitro* analyses. BLASTn searches against the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) confirmed unique amplification of *H. molitrix*, and no full matches to non-target taxa were detected. For *in vitro* testing, genomic DNA from related cyprinid species (*Barbonymus altus*, *B. gonionotus*, *Catlocarpio siamensis*, *Garra cambodgiensis*, *Paralaubuca riveroi*, *Parachela siamensis*, *Probarbus jullieni*, *Labeo rohita*, *Rasbora borapetensis*) and outgroup fish species (*Anabas testudineus*, *Pangasianodon hypophthalmus*, *Pangasianodon gigas*, *Chitala ornata*, *Channa micropeltes*, and *Oreoglanis siamensis*) was screened, and no amplification occurred in any non-target species, confirming 100% specificity.

Water parameter record

Environmental parameters were measured at each sampling location using calibrated field instruments. Temperature, pH, electrical conductivity, and total dissolved

solids (TDS) were recorded using a portable pH/temperature meter (Eutech PCST-estr–35, Singapore), while turbidity was measured using a turbidity meter (PONPE 509TU, Thailand). All measurements were taken from the same water column depth as eDNA samples to ensure data consistency and measured before eDNA collection at each site to ensure they reflected natural conditions at the time of sampling.

Statistical analysis

We employed Bayesian hierarchical occupancy models following Diana et al. (2021) to estimate site-level eDNA occurrence probability while accounting for imperfect detection at both the field sampling and laboratory stages. The modelling framework partitions uncertainty among three hierarchical components: site occupancy (ψ), sample-level eDNA availability given occupancy (θ), and qPCR detection probability given DNA presence in the extract (p). A low-probability false-positive parameter (α) was included to account for potential contamination or analytical artefacts, using the false-positive occupancy framework of Griffin et al. (2020) and Diana et al. (2021).

In this framework, ψ represents the probability that silver carp eDNA was truly present at a given site. Detection processes were modelled hierarchically, with θ describing the probability that eDNA was present in a collected water sample given site occupancy, and p representing the probability of detecting target DNA in a qPCR replicate given its presence in the extract. The dataset comprised 39 sites, each with three water samples per year and six qPCR replicates per sample.

Because water sampling was conducted during three discrete campaigns separated by two years (2019, 2021, and 2023), each year was modelled independently using a single-season false-positive occupancy framework implemented in the *eDNAoccupancy* R Shiny application (Diana et al. 2021). This approach ensured consistency with the assumptions of single-season occupancy models and avoided identifiability issues associated with sparse multi-year detection histories.

Covariate selection followed the Add–Delete–Swap (ADS) Bayesian variable-selection algorithm implemented in the *eDNAoccupancy* application. Turbidity was not included as a covariate because it is a highly transient, flow-dependent parameter and was not measured consistently across all sites and sampling years. To ensure model parsimony and comparability across surveys, only environmental variables recorded systematically across all sampling years (temperature, pH, electrical conductivity, and total dissolved solids, TDS) were considered. Posterior inclusion probabilities (PIPs) were used to quantify the strength of evidence for each predictor, and covariates with PIP ≥ 0.5 were retained in the final model following Diana et al. (2021). Regression coefficients were assigned weakly informative normal (0, 1) priors.

Model estimation was performed within the Shiny application using three Markov Chain Monte Carlo (MCMC) chains of 10,000 iterations each, with the first 5,000 iterations discarded as burn-in and thinning set to 2. Convergence was assessed using trace plots and the Gelman–Rubin statistic ($\hat{R} < 1.01$). Posterior means, 95% credible intervals, and posterior inclusion probabilities were used for inference. Full model equations, priors, and algorithmic details are provided in Suppl. material 2.

Negative field, extraction, and qPCR controls were included as validation data and consistently showed no amplification, constraining the estimated false-positive probability to near zero. In addition, rigorous laboratory precautions were imple-

mented, including physically separated pre- and post-PCR workflows and replicate-level detection criteria (at least one positive among six qPCR replicates within the LOD range), further minimising the likelihood of false-positive detections.

Data comparison with fisheries records

The eDNA results were compared with official DOF statistics obtained from the Fisheries Statistics of Thailand Annual Reports (Department of Fisheries Thailand 2019–2023). Data extraction focused on provincial–level aquaculture production and wild capture statistics for Chinese carps within the Chao Phraya River Basin provinces, specifically targeting Nakhon Sawan, Phichit, and Phitsanulok provinces. Statistical comparisons were conducted between eDNA detection rates and reported capture volumes, with temporal trends analysed using regression analysis. This comparison assessed the concordance between molecular detection patterns and traditional fishery–dependent monitoring approaches, while highlighting the limitations in species–specific resolution.

Results

Limit of detection (LOD) and limit of quantification (LOQ)

The detection thresholds for the *H. molitrix* qPCR assay were determined using a standard dilution series of synthetic DNA. Both the LOD and LOQ were estimated at 17.24 copies per reaction, corresponding to the lowest concentration at which ≥95% of replicates produced amplification with acceptable precision (coefficient of variation ≤35%). Detections were therefore classified as (i) quantifiable when ≥17.24 copies per reaction, (ii) below-quantification (bq) when consistently amplified but <17.24 copies per reaction, or (iii) non-detections when no amplification occurred in any replicate. This framework was used to categorise all site-level qPCR results and is reflected in Table 1. No amplification of *H. molitrix* DNA occurred in any field, extraction, or qPCR negative control, confirming that all detections reported in this study originated from environmental samples.

eDNA detection patterns

Environmental DNA detection rates demonstrated substantial increases across the three–year study period, indicating an expanding presence of *H. molitrix* throughout the Chao Phraya River Basin (Table 1 and Fig. 2). In 2019, *H. molitrix* eDNA was detected at 12 of 39 sites (31%), with 11 sites showing below–quantification signals and 16 sites yielding no detectable DNA. Detection success improved markedly by 2021, with positive detections recorded at 13 sites (33%) and below–quantification detections at 21 sites (54%), leaving only 5 sites (13%) without detectable eDNA. Notably, by 2023, positive detections had reached 23 sites (59%), with all remaining 16 sites showing below-quantification (bq) signals; no sites remained completely negative for *H. molitrix* eDNA.

River–specific patterns revealed distinct temporal dynamics. The Ping River showed no quantifiable detections in 2019 or 2021, with all results remaining at below–quantification levels. By 2023, sites 6 and 8 exceeded quantification thresholds (18.99 and 19.71 copies per reaction, respectively).

Table 1. Summary of silver carp eDNA concentrations across 39 sampling sites in the Chao Phraya River Basin, Thailand, over three sampling years (2019, 2021, and 2023).

Site	River	eDNA concentration (copies per reaction)					
		2019	detection	2021	detection	2023	detection
1	Ping	0	nd	4.62	bq	9.43	bq
2		0	nd	2.83	bq	5.56	bq
3		11.79	bq	9.79	bq	12.89	bq
4		8.95	bq	9.02	bq	15.08	bq
5		0	nd	7.83	bq	12.52	bq
6		0	nd	9.72	bq	18.99	+
7		8.50	bq	8.50	bq	15.53	bq
8		8.89	bq	9.94	bq	19.71	+
9	Wang	3.98	bq	0	nd	5.77	bq
10		17.49	+	10.01	bq	13.28	bq
11		17.89	+	9.50	bq	12.15	bq
12		17.24	+	9.79	bq	14.85	bq
13		7.55	bq	9.09	bq	16.36	bq
14		7.77	bq	9.86	bq	16.61	bq
15		10.01	bq	9.79	bq	20.15	+
16		0	nd	9.72	bq	19.13	+
17	Yom	0	nd	0	nd	5.60	bq
18		0	nd	0	nd	3.21	bq
19		19.13	+	24.27	+	82.88	+
20		86.66	+	95.47	+	177.06	+
21		22.20	+	18.57	+	50.71	+
22		19.85	+	18.02	+	35.22	+
23		4.45	bq	16.24	bq	44.35	+
24		2.07	bq	17.24	+	48.14	+
25	Nan	0	nd	0	nd	1.59	bq
26		34.18	+	20.15	+	38.22	+
27		10.79	bq	145.91	+	46.73	+
28		0	nd	0	nd	13.28	bq
29		0	nd	16.86	bq	35.48	+
30		0	nd	16.48	bq	33.18	+
31		0	nd	12.06	bq	39.37	+
32		0	nd	11.62	bq	54.63	+
33	Chao Phraya	0	nd	15.88	bq	31.97	+
34		0	nd	20.61	+	29.90	+
35		0	nd	219.71	+	34.44	+
36		50.71	+	89.28	+	203.95	+
37		389.70	+	258.8	+	401.48	+
38		192.16	+	92.67	+	173.15	+
39		183.77	+	95.47	+	183.77	+

+ = Positive detection (above limit of quantification, ≥17.24 copies per reaction)
bq = Below quantification (detectable but <17.24 copies per reaction)
nd = No detection (no amplification)
LOD/LOQ = 17.24 copie per reaction

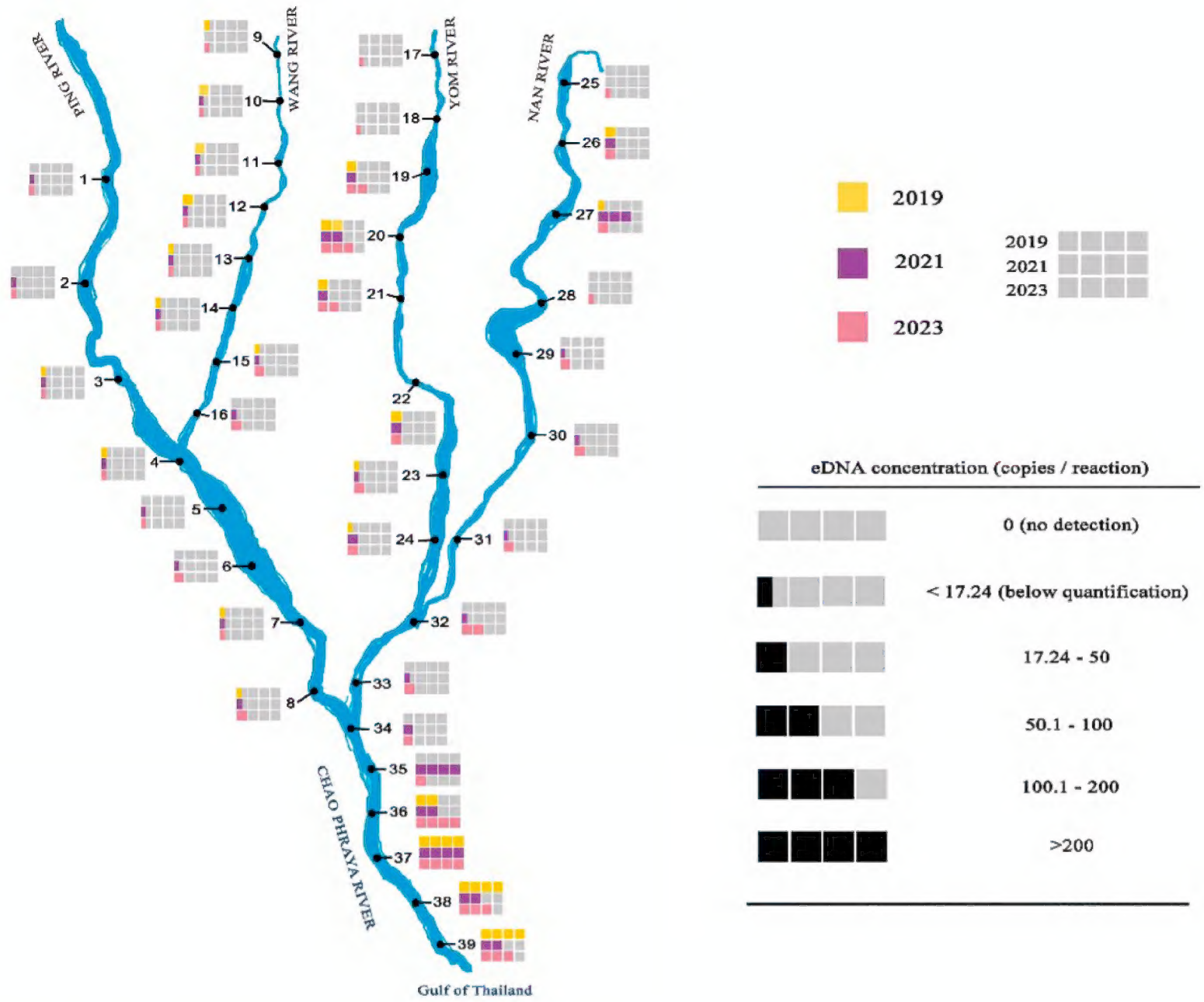

Figure 2. eDNA detection results across 39 sampling sites in the Chao Phraya River Basin, Thailand, during 2019, 2021, and 2023.

The Wang River showed early quantifiable eDNA concentrations at sites 10 and 11 in 2019 (17.49 and 17.89 copies per reaction), but these values declined to below the quantification threshold in subsequent years. Conversely, sites 15 and 16 showed increasing concentrations by 2023 (20.15 and 19.13 copies per reaction).

The Yom River demonstrated the most consistent and substantial eDNA signals among tributary systems. Site 20 maintained the highest concentrations throughout the study period, increasing from 86.66 copies/reaction in 2019 to 177.06 in 2023. Multiple sites (19, 21, 22) exceeded quantification thresholds in 2019 and showed dramatic increases by 2023.

In the Nan River, site 26 showed persistent quantifiable concentrations in 2019 and 2023 (34.18 and 38.22 copies per reaction), while site 27 exhibited the most dramatic fluctuation, peaking at 145.91 copies per reaction in 2021 before declining to 46.73 in 2023. Several sites (31, 32) exceeded quantification thresholds by 2023.

The main Chao Phraya River consistently yielded the strongest eDNA signals throughout the study period. Site 37 recorded the highest concentrations across all locations and years, maintaining exceptional levels with 389.70 copies per reaction in 2019 and peaking at 401.48 in 2023. Sites 36, 38, and 39 also showed consistently high concentrations (Table 1).

Occupancy modelling results

Bayesian hierarchical occupancy models provided robust estimates of silver carp eDNA occurrence probabilities and detection probabilities, interpreted as evidence

Table 2. Bayesian posterior mean estimates of occurrence probability (ψ) and detection probabilities for *H. molitrix* across three sampling years.

Year	Occurrence (ψ)	Probability of true positives		Probability of false positives	
		Field (θ_{11})	Lab (p_{11})	Field (θ_{10})	Lab (p_{10})
2019	0.5088	0.9000	0.9000	0.1001	0.1001
2021	0.3961	0.8997	0.9000	0.1000	0.1001
2023	0.4130	0.9000	0.8999	0.1000	0.1000

of species presence while accounting for observation uncertainty at multiple stages (Table 2). Across the three sampling years, estimated site-level occupancy probabilities (ψ) remained moderate but consistent, ranging from 0.396 in 2021 to 0.509 in 2019. The slight decline in 2021 followed by stabilisation in 2023 (0.413) may reflect temporal variation in detectability or localised population dynamics rather than systematic range contraction.

Detection probabilities remained consistently high across both field sampling and laboratory analysis stages. True-positive detection probabilities in field sampling (θ_{11}) ranged from 0.8997 to 0.9000 across all years, while laboratory-based true-positive detection (p_{11}) showed similar consistency (0.8999 to 0.9000). These high values indicate that when silver carp eDNA was present in environmental samples, it was reliably detected through both observation stages.

False-positive probabilities remained very low across all detection stages, with field-level false positives (θ_{10}) ranging from 0.1000 to 0.1001 and laboratory false positives (p_{10}) from 0.1000 to 0.1001. These results confirm minimal observation error and high reliability of the multi-stage eDNA detection framework.

Bayesian variable selection and regression coefficients indicated that environmental effects on occupancy and detection were year-specific and parameter-dependent (Table 3; Suppl. material 1: table S2). Across all three sampling years, no environmental covariate showed a supported effect on site occupancy (ψ), with all PIPs below 0.5 and regression coefficient credible intervals overlapping zero.

At the laboratory level, effects on true detection probability (p_{11}) varied among years. In 2019, conductivity showed a supported negative effect on p_{11} (posterior mean $\beta = -0.040$, 95% credible interval: −0.073 to −0.007), whereas temperature effects were weak and uncertain. In 2021 and 2023, no environmental covariate exhibited a supported directional effect on p_{11}.

For laboratory false-positive probability (p_{10}), temperature effects differed through the years. In 2023, temperature had a strong positive effect on p_{10} (posterior mean $\beta = 0.041$, 95% credible interval: 0.023 to 0.055; PIP = 0.9996), indicating increased false-positive probability at higher temperatures. In contrast, temperature effects on p_{10} were weak and unsupported in 2019 and 2021. No other environmental covariate showed a supported directional effect on p_{10} in any year.

At the field level, no environmental covariate exhibited a supported directional effect on either false-positive (θ_{10}) or true detection probability (θ_{11}) across years, despite moderate posterior inclusion probabilities for some variables. All regression coefficient credible intervals overlapped zero, indicating limited evidence for systematic environmental control of field-level detection processes.

Overall, these results indicate that environmental conditions influenced eDNA detection processes in a limited and context-dependent manner, whereas site-level eDNA occurrence (ψ) remained comparatively stable across years. The generally

Table 3. Posterior inclusion probabilities (PIPs) for environmental covariates as predictors of eDNA detection probability (ψ), interpreted as evidence of presence and detection parameters (θ_{11}, θ_{10}, p_{11}, p_{10}). Values in bold indicate influential covariates (PIP ≥ 0.5).

Covariate	Year	pH	Temperature	Conductivity	Total dissolved solids
ψ	2019	0.4228	0.3626	0.4190	0.4342
	2021	0.3954	0.3928	0.2516	0.2566
	2023	0.3852	0.3748	0.2746	0.2756
p_{10}	2019	**0.6076**	**0.6970**	**0.6026**	**0.7132**
	2021	**0.5376**	**0.6176**	0.4946	**0.5702**
	2023	0.4540	**0.9996**	0.1398	0.1562
p_{11}	2019	0.4998	**0.7920**	**0.6834**	**0.5734**
	2021	**0.5702**	**0.6910**	**0.7208**	**0.8052**
	2023	0.4388	0.4438	**0.8078**	**0.8118**
θ_{10}	2019	0.4986	**0.5556**	0.4366	0.4298
	2021	**0.5682**	**0.5728**	**0.6446**	**0.6160**
	2023	**0.6274**	**0.6272**	**0.7602**	**0.7212**
θ_{11}	2019	**0.7316**	**0.7406**	**0.7382**	0.2728
	2021	0.4186	**0.6356**	0.3898	0.4162
	2023	0.3746	**0.6912**	**0.6958**	0.3806

stable ψ values over time, together with high and stable p estimates and very low false-positive probabilities, provide further evidence that silver carp eDNA has become widely detectable across the basin, consistent with an ongoing pattern of range expansion and establishment.

Comparison with fisheries data

Department of Fisheries records from the National Statistical Office confirmed substantial aquaculture production of Chinese carps in key provinces within the Chao Phraya Basin, specifically Nakhon Sawan Province (central basin location), Phichit Province (lower basin), and Phitsanulok Province (northern basin), with consistent annual production documented throughout the study period. However, capture fishery data from natural systems showed declining trends, decreasing from 1,273 to 1,055 metric tons between 2019 and 2023.

Spatial comparison revealed notable discrepancies between eDNA detection patterns and traditional fishery records. Several upstream sampling sites with positive eDNA detections lacked corresponding capture fishery records. Conversely, some provinces with large–scale aquaculture production showed limited eDNA signals at nearby sampling sites. The eDNA approach provided markedly superior spatial resolution and species–specific information compared to aggregated fishery statistics (Table 4).

Discussion

H. molitrix range expansion and ecological implications

This study provides comprehensive molecular evidence for the expanding presence and distribution of *H. molitrix* in Thailand's most important freshwater system.

Table 4. Comparison of eDNA detection patterns with DOF aquaculture and capture statistics for Chinese carps (*H. molitrix* and *H. nobilis*).

Year	eDNA Monitoring			DOF Statistics		
	Sites with detectable eDNA	Quantifiable detections	Detection rate	Aquaculture production*	Wild capture	Total production
	(n/39)	(% positive)	(% total)	(tonnes)	(tonnes)	(tonnes)
2019	28	12 (30.8%)	72%	462	1,273.6	1,735.6
2021	34	13 (33.3%)	87%	157	1,230.3	1,387.3
2023	39	23 (59.0%)	100%	208	1,055.2	1,263.2

The presence of *H. molitrix* eDNA increased from 31% of samples in 2019 to 59% of samples in 2023, with detectable DNA found at all sampling sites by the final survey year, demonstrating clear evidence of range expansion and potential population growth throughout the Chao Phraya River Basin. This temporal progression from sporadic detection to basin–wide presence suggests both geographic expansion and potential population growth of *H. molitrix* throughout the study area. The systematic increase in quantifiable detections, particularly at downstream locations and major confluences, aligns with expected dispersal patterns for this highly mobile species in connected river networks.

It is also possible that downstream sites exhibited higher eDNA concentrations due to hydrological transport and aggregation of genetic material from upstream populations. Such downstream accumulation has been observed in other large river systems (Carraro et al. 2018; Laporte et al. 2020) and should therefore be considered when interpreting elevated detection frequencies in the main Chao Phraya River, which may reflect both true biological presence and passive transport processes.

Before interpreting these spatial and temporal patterns, it is important to clarify the ecological meaning of eDNA detections in this study. All detections reported here are based on environmental DNA rather than direct captures of fish. In riverine systems, eDNA can be transported downstream or diluted across hydrological gradients, and therefore a detection does not necessarily indicate the immediate presence of individuals at the exact sampling point. However, repeated detections of silver carp eDNA at the same sites across multiple years provide strong evidence that these habitats are persistently used or occupied by the species. The consistency of eDNA signals through time, together with the increasing basin-wide detection probability observed in this study, supports the inference that silver carp are now widespread in the Chao Phraya River Basin. This interpretation acknowledges the potential for downstream transport of eDNA but also highlights its power to reveal species presence patterns that would be difficult to document through conventional sampling across such an extensive and dynamic river system.

These findings are consistent with invasion patterns documented in other regions where *H. molitrix* has formed self–sustaining populations with notable ecological impacts (Irons et al. 2007; Coulter et al. 2016). The river–specific patterns observed in this study offer valuable insights into colonisation dynamics. Recent establishment or growth was evident in the Ping River, spatial redistribution occurred within the Wang River, populations in the Yom River appeared well–established, continued expansion was observed in the Nan River, and widespread presence was confirmed in the main Chao Phraya River channel. Together, these patterns reflect a comprehensive basin–wide colonisation process.

From a management perspective, the universal eDNA detectability achieved by 2023 suggests that silver carp has reached a critical threshold for potential eco-

system impact throughout the Chao Phraya River Basin. Studies from established populations demonstrate that silver carp populations can dramatically alter plankton community structure, reduce water clarity, and competitively displace native planktivorous species once sufficient densities are achieved (Zhang et al. 2016). The widespread distribution documented in our study suggests that preventive management opportunities may have been missed, necessitating a shift to containment and impact mitigation strategies.

Methodological reliability and environmental factors

The application of Bayesian occupancy modelling frameworks enabled robust separation of true eDNA occurrence probability from detection-related processes, addressing a critical challenge in eDNA-based biodiversity assessments (Hunter et al. 2017; Dorazio and Erickson 2018). The moderate but consistent eDNA occurrence probability estimates (ψ = 0.40–0.51) suggest that while silver carp has not achieved complete basin-wide occupancy, its distribution has become increasingly widespread and persistent across diverse habitat types, consistent with the environmental tolerance characteristics of this cyprinid species (Jennings 1988; Kolar et al. 2005). The slight decline in 2021, followed by stabilisation in 2023, may reflect temporal variation in detectability or localised population dynamics rather than systematic range contraction.

Although nearly all sites showed detectable *H. molitrix* eDNA by 2023, the model-estimated occupancy probabilities ($\psi \approx$ 0.4–0.5) were lower than the raw detection frequency. This difference reflects the hierarchical structure of the Bayesian occupancy model, which adjusts for imperfect detection and the uncertainty of weak or below-quantification (bq) signals. The "bq" detections represent concentrations near the analytical limit of detection, where stochastic amplification effects increase the probability of both false negatives and false positives (Hunter et al. 2017). Consequently, the model assigns reduced weight to such uncertain detections, resulting in ψ values lower than 1 even when all sites yield at least minimal signal. In other words, ψ expresses the probability that *H. molitrix* are truly present at a site after accounting for measurement uncertainty, rather than the simple proportion of observed positives. This conservative estimation framework reduces over-confidence in occupancy inference from trace-level eDNA detections.

The consistently high laboratory detection probabilities ($p_{11} \approx 0.90$) demonstrate the technical reliability of the species-specific qPCR assay under tropical environmental conditions. High detection probabilities also indicate that environmental sampling protocols effectively captured target DNA when it was present. The minimal observation error and very low false-positive probabilities confirm the robustness of the multi-stage eDNA detection framework.

Although multi-season occupancy models (Royle and Kéry 2007) or single-season models incorporating year as a covariate can provide a formal structure for estimating colonisation and extinction probabilities, our analysis was designed around independent single-season frameworks. The *eDNAoccupancy* application (Diana et al. 2021) used in this study currently supports single-season formulations only, and each sampling campaign (2019, 2021, and 2023) represented a discrete field effort separated by two years. Conducting independent models for each year therefore maintained consistency with the data structure and ensured model identifiability while remaining within the validated Griffin et al. (2020)

false-positive occupancy framework. Future monitoring that incorporates annual or higher-frequency sampling could allow the application of multi-season models to explicitly quantify temporal transitions in occupancy across years.

The Bayesian variable-selection framework applied in this study (Diana et al. 2021) provided a transparent means of identifying environmental predictors influencing eDNA occupancy (ψ) and detection processes. Across all sampling years, none of the evaluated environmental covariates exhibited supported effects on site-level occupancy (ψ), indicating that the probability of *H. molitrix* eDNA occurrence was relatively stable across a wide range of environmental conditions. This stability in ψ is consistent with the broad ecological tolerance and invasive capacity of the species (Kolar et al. 2005).

In contrast, environmental effects were observed primarily within detection submodels and were both parameter- and year-specific. Temperature showed no supported effect on true detection probability (p_{11}) in any year but exhibited a positive effect on laboratory false-positive detection probability (p_{10}) in 2023, indicating increased apparent detection at higher temperatures under certain conditions. Other water-quality variables (pH, conductivity, and TDS) showed weak or inconsistent effects on detection and were not associated with supported directional effects across years. Although temperature is widely recognised as an important driver of eDNA dynamics through its influence on DNA degradation and microbial activity (Strickler et al. 2015), our results suggest that its effects on detectability in this tropical river system were limited and context dependent rather than uniformly reducing true detection.

Collectively, these findings indicate that environmental conditions exerted a relatively minor influence on site-level eDNA occurrence, while detection processes were affected in a nuanced and temporally variable manner. The combination of moderate and stable ψ estimates, consistently high true detection probabilities, and very low false-positive rates supports the robustness of the eDNA-based framework and is consistent with the widespread establishment of *H. molitrix* across the Chao Phraya River Basin.

Comparison with traditional monitoring and management implications

Comparison between eDNA results and official fishery statistics revealed fundamental limitations in traditional monitoring approaches for introduced species surveillance, particularly in developing countries where taxonomic expertise and standardised reporting frameworks remain limited (Minamoto et al. 2012; Rees et al. 2014; Deiner et al. 2017). In Thailand, DOF records document substantial Chinese carp aquaculture production and some wild capture throughout the basin, but the continued aggregation of *H. molitrix* and *H. nobilis* under collective reporting categories precludes species-specific assessment. In addition, the coarse spatial resolution of fishery statistics at the provincial level limits their utility for site-level management and early detection of range expansion.

In contrast, eDNA monitoring provided fine-scale, species-specific resolution and detected *H. molitrix* presence at multiple upstream sites where no recent capture records exist. This illustrates how molecular surveillance can complement traditional fisheries assessments by revealing spatial patterns that are not discernible from aggregated or provincial-scale statistics. The detection of *H. molitrix* eDNA at locations lacking corresponding fishery records suggests either natural dispersal

from undocumented source populations or escapement from small-scale aquaculture operations not captured in official statistics, highlighting potential gaps in current biosecurity and reporting frameworks.

Conversely, some provinces with large-scale aquaculture production exhibited limited eDNA signals at nearby sampling sites, which may reflect effective containment practices, hydrological dilution in high-flow river reaches, or temporal mismatches between sampling and production cycles. Overall, the ability of eDNA monitoring to detect species presence at sites with no traditional capture records underscores its value as a sensitive and complementary tool for early detection and spatial risk assessment of introduced species.

The integration of eDNA monitoring into Thailand's aquatic biodiversity surveillance frameworks would substantially enhance national capacity for managing introduced species. Molecular surveillance could be strategically prioritised in areas adjacent to aquaculture facilities or river reaches with high hydrological connectivity to farming operations, where escape risk and establishment probability are elevated. Regulatory agencies could use eDNA evidence to inform licensing requirements, spatial zoning policies, and mitigation measures for carp aquaculture, particularly in ecologically sensitive watersheds.

Conclusions

This study demonstrates the effectiveness of eDNA monitoring for tracking the distribution of *H. molitrix* across Thailand's largest watershed, revealing widespread species presence across multiple river reaches. Bayesian occupancy modelling confirmed the technical robustness of the molecular surveillance framework, with consistently high true detection probabilities and very low false-positive rates across sampling years. Environmental effects on eDNA detection were limited and context dependent, with no supported influence on site-level occupancy and only year-specific effects on laboratory detection processes.

Comparison with traditional fishery statistics highlighted key advantages of molecular surveillance, particularly its ability to provide species-specific and site-level information that is not attainable from aggregated catch records. Taken together, these findings support the integration of eDNA monitoring into national aquatic biodiversity surveillance and biosecurity frameworks and demonstrate its value as a complementary tool for managing introduced species. The methodological framework presented here offers a transferable template for aquatic species monitoring across Southeast Asia, where aquaculture expansion and limited reporting resolution continue to challenge effective management.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

All field sampling procedures were conducted in accordance with current laws in Thailand on environmental research and were approved by the safety management committees for experiments: Protocol Number 2561/FA-0001, Protocol Number 640204004, and Protocol Number 2566/FA-0002. The study followed the recommendations in the ARRIVE guidelines for environmental DNA research. All water sampling was conducted using non-invasive methods that did not require direct capture or handling of aquatic organisms.

Use of AI

During the preparation of this work the authors used QuillBot in order to improve readability and language. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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Author contributions

MO: Conceptualisation, Methodology, Software, Investigation, Formal analysis, Resources, Data Curation, Visualisation, Funding acquisition, Writing – Original draft, Writing – Review and Editing. KO: Software, Data Curation, Writing – Review and Editing. SO: Software, Data Curation, Writing – Review and Editing. TJ: Supervision, Writing – Review and Editing. CS: Resources, Writing – Review and Editing.

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Data availability

The datasets generated and analysed during this study are available within the article or its supplementary materials. Detailed laboratory protocols and primer sequences are included in the methods section.

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Supplementary material 1

Geographic coordinates and river system assignment for the 39 eDNA sampling sites in the Chao Phraya River Basin

Authors: Maslin Osathanunkul, Khukrit Osathanunkul, Sarawut Ounjai, Tuantong Jutagate, Chatmongkon Suwannapoom

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Supplementary material 2

Bayesian occupancy model structure

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